

Effect of Alkali Pretreatment on Water Hyacinth Biomass for Production of Ethanol

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Abstract

The current crisis of fossil fuels, a non-renewable resource, has become a serious issue to be taken care of in our modern world. Bioethanol from lignocellulosic biomass is a potential alternative to the conventional fossil fuels. The structural and chemical complexity of the biomass provides a significant hindrance to the production of bio-ethanol. However, various chemical and biological pretreatment methods can break the complex structure of the biomass to produce various sugar derivatives. They are further treated by enzymatic methods to increase the yield of reducing sugars significantly, and finally fermented with the help of microorganisms to produce bioethanol. In enzymatic saccharification process, maximum yield for reducing sugars was 465 mg/g and xylose was 231 mg/g using commercial enzymes cellulase and xylanase. Ethanol production is maximum with *Pichia stipitis* (4.13 gm/L) as compared to *Candida shehatae* (3.79 gm/L) and *Saccharomyces cerevisiae* (3.07 gm/L). This paper emphasizes the importance of water hyacinth in the production of bioethanol and the effect of alkali pretreatment method on the biomass and the future scope.

Keywords

Water Hyacinth Biomass; Lignocellulose; Alkali Pretreatment; Bioethanol; Fermentation

Introduction

The present scenario of the nature and its resources has resulted in the scientific attraction in using biological renewable resources as an alternative to the use of limited resources rapidly depleted such as fossil fuels. Lignocellulosic biomass can be used as an alternative to the automobile fuels due to its availability, abundance and relatively low cost.

Lignocellulosic biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its

chemical bonds (McKendry, 2002). It has been reported that ethanol produced from lignocellulosic biomass resources has the potential to cut greenhouse gas emissions by 86% as it is burnt more completely as compared to petrol. Ethanol has a much higher latent heat of vaporization (855 MJ/kg) than petrol (293 MJ/kg) as well as a higher octane number (99) than petrol (80–100) and, as a result, pre-ignition does not occur when ethanol is used (Ganguly et al, 2012). Moreover, it makes no net contribution to the global warming because the carbon dioxide produced by the combustion of ethanol is consumed during plant growth which continues the carbon cycle balance in the nature.

Water hyacinth (*Eichhornia crassipes*), a widely prevalent aquatic weed in India, as a potential biomass resource for various uses, whose high hemicellulose content (30–55% of dry weight) can provide hemicellulosic sugars for bioconversion to ethanol fuel, is considered as a noxious weed in many parts of the world as it is an exceptionally fast growing plant and depletes nutrients and oxygen rapidly from water bodies, adversely affecting flora and fauna. Hence, this tropical plant can cause infestation over large areas of water, leading to a series of problems like reduction of biodiversity, blockage of rivers and drainage system, depletion of dissolved oxygen, alteration of water chemistry and involvement in environmental pollution. Moreover, due to high evapotranspiration, it incurs crisis all over the places where it grows.

Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin as well as other minor components such as proteins, oils, and ash, etc. The carbohydrate polymers (cellulose and hemicelluloses) are tightly bound to the lignin, by hydrogen and covalent bonds, which results in complex structure that provides a hydrolysis-resistant protecting sheet

around the cellulose. Cellulose, the principle component of lignocellulosic biomass, whose concentration ranges from 40 to 45% of dry weight (Sjöström, 1993), is a linear polymer of D-glucose subunits that forms cellobiose units by β -(1, 4)-glycosidic linkage. Hemicellulose concentration in lignocellulosic biomass is 25 to 35% and it is easily hydrolysable to fermentable sugars (Saha, 2007). The chemical structure of hemicellulose consists of long chains of a variety of pentoses, hexoses, and their corresponding uronic acids such as D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acid which are linked together by β -1, 4- and occasionally β -1, 3-glycosidic bonds. Lignin, the second most abundant biological material on the planet, comprises about 15-25% of dry weight of woody plants. The chemical structure of lignin is very complicated and based on three monomeric precursors: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol. It acts as a cementing agent by preventing the invasion of the destructive enzymes through the cell wall and thus playing an important role in plant defense mechanism.

Alkaline pretreatment is basically a delignification process, in which a significant amount of hemicellulose is solubilised as well. The action mechanism is believed to be the saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components. It also removes acetyl and various uronic acid substitutions on hemicellulose that reduce the accessibility of hemicellulose and cellulose to enzymes (Sun et al, 2002). Alkaline pretreatment of lignocellulosic materials causes swelling, leading to decreased degree of polymerization and crystallinity, increased internal surface area, disruption of lignin structure, and separation of structural linkages between lignin and carbohydrates (Fan et al, 1987).

Enzyme saccharification is carried out with the help of cellulase and xylanase. The synergistic effect provided by the enzymes gives a better yield of reducing sugars (Kumar and Wyman, 2009).

Many organisms develop without using the electron transport chain. The generation of energy without the electron transport chain is called fermentation. This definition is the exact and original meaning of the term fermentation, although currently it is often used in a broader context (Shuler and Kargi, 1992). Microorganisms i.e. yeast like *Pichia stipitis*, *Candida*

shehatae, *Saccharomyces cerevisiae* etc. follow the above metabolism pathways to produce ethanol.

Materials and Methods

Chemicals, Reagents and Microorganisms

All chemicals used were of analytical grade and commercially available. The organisms *Saccharomyces cerevisiae*, *Pichia stipitis*, *Candida shehatae* were maintained at 4°C in Sabouraud Dextrose Agar (10g/L neo-peptone, 20 g/L dextrose, pH 6.5; SDA). Enzymes cellulase and xylanase were commercially available from Sigma-Aldrich.

Preparation of Water Hyacinth Biomass

Fresh water hyacinth biomass (WHB) was collected from the ponds inside the campus of CSIR-C.M.E.R.I. The samples were washed thoroughly and carefully to remove every bits of dust and dirt from them and were further chopped and blended to very small particles (~2mm) and finally dried at 106°C for about 6 hrs. The dried biomass was stored in air tight containers at room temperature.

Alkali Pretreatment

The pretreatment process was carried out by using 5% NaOH (1 g sample+10 mL of 5% NaOH). The mixture of biomass and alkali was allowed to soak for 1 h followed by the treatment time of 10 mins at 150°C inside the furnace. The hydrolysate was collected by filtration method.

Enzyme Saccharification

For better yield of reducing sugars, the hydrolysate was exposed to enzyme saccharification. Enzymatic saccharification of acid treated WHB was carried out in reaction mixture containing treated biomass, Mandel media [The Mandel's medium was prepared using the following composition (g/L) 10.0 g; urea, 0.3; peptone, 0.75; yeast extract, 0.25; (NH₄)₂SO₄, 1.4; KH₂PO₄, 2.0; CaCl₂, 0.3; MgSO₄.7H₂O, 0.3 and trace elements (mg/l): FeSO₄.7H₂O, 5; MnSO₄. 4H₂O, 1.6; ZnSO₄.7H₂O, 1.4 and CoCl₂.6H₂O, 20.0 (Mandels et al., 1976)] and enzymes cellulase (103.75 U/g) and xylanase (650.18 U/g) dissolved in citrate buffer. The reaction mixture was incubated on a rotatory shaker adjusted to 50°C and 75 rpm. The samples were withdrawn at intervals of 12 h up to 60 h for reducing sugar and xylose content determination. After complete saccharification, the reaction mixture was

heated slightly for deactivation of the enzymes. Finally, the saccharified biomass was filtered using filter paper and the hydrolysate thus the acquisition was used for fermentation.

Fermentation

For fermentation, the yeast *Pichia stipitis*, *Candida shehatae* and *Saccharomyces cerevisiae* were used. From the fully cultured plates, a loop full of culture of each was transferred into autoclaved broth medium [broth medium for the culture of *Pichia stipitis* and *Candida shehatae* was prepared with the following composition (g/L) 50 mL: D-xylose, 50; Glucose, 5; Yeast extract, 3; Malt extract, 3; Peptone, 5; pH, 5.0; broth medium for the culture of *Saccharomyces cerevisiae* was prepared with the following composition (g/L) 50 mL: d-glucose, 10; Peptone, 20; Yeast extract, 10; pH, 5.0] separately for each under laminar air flow. They were cultured for about 20 h in shaker incubator. The broths were then centrifuged at about 10,000 rpm for 10-15 min. The supernatant for each was discarded and the pellets containing the yeast cells were collected and added to sterilized hydrolysate obtained after saccharification. Samples from each flask were collected and estimated for yield of ethanol at a time interval of 1 h, 2 h and thereafter at a regular interval of 2 h up to when the yield of ethanol became almost stagnant.

Determination of different sugar contents was done by Tollen's test (for xylose) (Bartos and Pesez (1979)) and DNSA assay (for reducing sugar) (J. Bartos, M. Pesez (1979)). The ethanol yield was estimated by means of the dichromate assay (Archer et. al., 2007).

Results and Discussion

Sugar Yeild from Water Hyacinth Biomass

WHB was autohydrolysed at high temperature (~110°C) inside the furnace, and the obtained xylose yield is shown in Table 1.

TABLE 1 AVERAGE YIELD OF XYLOSE FROM AUTOHYDROLYSED WATER HYACINTH BIOMASS

SUGAR YEILD (mg/g)	READING 1	READING 2	READING 3	MEAN
Xylose	54.6	58.3	56.9	56.6

Sugar Yeild from Alkali Hydrolysis of Water Hyacinth Biomass

When WHB was soaked in sodium hydroxide for 1 hour and then treated at a high temperature (150°C)

inside the furnace, the obtained xylose yield was as shown in Table 2.

Fig.1 shows that the xylose yield from alkali-hydrolysed WHB (Xylose = 25.8 mg/g) is lesser than that from the auto hydrolysed WHB (Xylose=56.6 mg/g). However, alkali pretreatment is preferred over autohydrolysis process as it removes lignin and solubilises a small part of the sugar. Hence, the yield of sugar during saccharification increases.

TABLE 2 AVERAGE YIELD OF XYLOSE FROM ALKALI TREATED WATER HYACINTH BIOMASS

SUGAR YEILD (mg/g)	READING 1	READING 2	READING 3	MEAN
Xylose	26.3	25.8	25.4	25.8

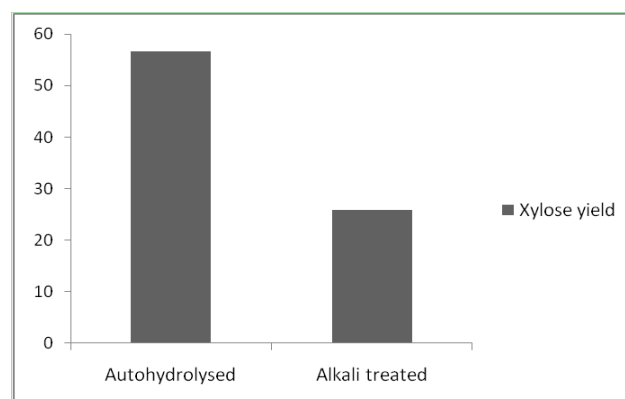


FIG. 1 A COMPARATIVE GRAPH BETWEEN AUTOHYDROLYSED WHB AND ALKALI-HYDROLYSED WHB

Enzyme Saccharification of Alkali Treated Biomass

Pre-treated WHB was further treated with cellulase and xylanase together after washing and drying. Their synergistic action gave the maximum sugar yield as shown in Fig. 2.

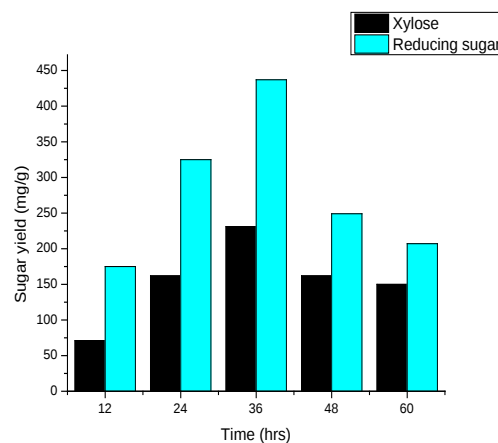


FIG. 2 SUGAR YIELD FROM WATER HYACINTH BIOMASS UPON ENZYME SACCHARIFICATION

Maximum amount of sugar was yielded (reducing sugars=465 mg/g and xylose=231 mg/g) with 103.75 U/g of cellulase and 650.18 U/g of xylanase for 36 hrs. The amount of the sugar yielded after enzyme saccharification is much higher than that of simple alkali hydrolysis, which explains the significance of enzyme saccharification.

Estimation of Ethanol Yield

The saccharified hydrolysate of WHB with the highest yield of sugars was taken for fermentation. Each microorganism species, *Pichia stipitis*, *Candida shehatae*, *Saccharomyces cerevisiae* was cultured and inoculated in the hydrolysate separately. They were allowed to ferment for about 16 h. The results are shown in Fig. 3.

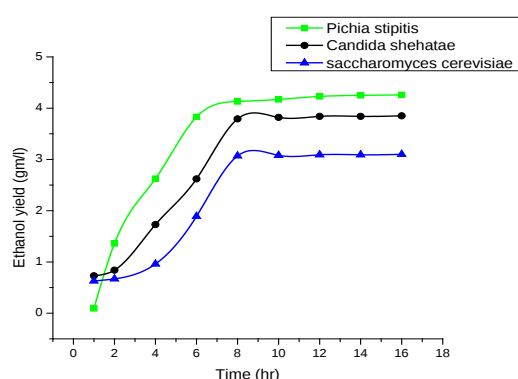


FIG. 3 ETHANOL YIELD FROM VARIOUS YEAST CULTURE AT DIFFERENT TIME INTERVAL

The highest yield for each was obtained after almost 8 h, after which the increase in yield was almost negligible. The ethanol obtained after about 8 h of fermentation was:

Pichia stipitis = 4.13 g/L

Candida shehatae = 3.79 g/L

Saccharomyces cerevisiae = 3.07 g/L

Clearly from the above readings, it can be concluded that *Pichia stipitis* gives the highest yield of ethanol as it can degrade pentoses as well as hexoses.

Conclusion

The technique is helpful in lowering the population of water hyacinth while providing a simple and low cost process, which will be economically feasible for the developing countries. Based on different analyses of sugar content, it has been observed that the sugar yield (xylose) is lesser after alkali treatment (Xylose-25.8 mg/g) as compared to the autohydrolysed biomass (Xylose-56.6 mg/g). However, alkali treatment

is preferred over autohydrolysis process for further treatments because it mainly used for delignification of the biomass and solubilises small amount of reducing sugars. Hence major part of the sugar can be utilised for saccharification. In enzyme saccharification process, maximum yield for reducing sugar was 465 mg/g and xylose was 231 mg/g, which implies that with the use of commercial enzymes cellulase and xylanase, the sugar yield is maximum as compared to the crude enzyme. Ethanol production was maximum with *Pichia stipitis* (4.13 g/L) as compared to *Candida shehatae* (3.79 g/L) and *Saccharomyces cerevisiae* (3.07 g/L), as it can degrade pentoses as well as hexoses. Thus, from water hyacinth, a noxious weed, a reasonable amount of ethanol can be produced to be used as an alternative biofuel in near future.

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